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In order for meaningful comparisons to be made between experiments from different laboratories, reliable dosimetry is needed for biological systems exposed to microwave radiation. We present an improved analytical method for determining energy absorption which uses heating and cooling curves. The method is demonstrated for in vitro samples that were exposed to microwave radiation in two different exposure systems. Advantages and disadvantages of the method, as well as practical considerations, are discussed.

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Measurement of microwave radiation absorbed by biological systems, 1, Analysis of heating and cooling data

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In order for meaningful comparisons to be made between experiments from different laboratories, reliable dosimetry is needed for biological systems exposed to microwave radiation. We present an improved analytical method for determining energy absorption which uses heating and cooling curves. The method is demonstrated for *in vitro* samples that were exposed to microwave radiation in two different exposure systems. Advantages and disadvantages of the method, as well as practical considerations, are discussed.

1. INTRODUCTION

Scientists investigating biological effects of microwave radiation are confronted with a major problem: the development of dosimetric methods that provide a reliable basis for comparison of data. Although pleas have been made repeatedly [*Justesen and King*, 1970; *Guy*, 1975; *Johnson*, 1975] that dose data be reported in terms of energy or rates of energy absorbed by the sample, many workers are still ignoring this issue. In much of the reported work only estimates of the power density in the far field are provided, and no attempt has been made to estimate or measure the absorbed energy. In other experiments where exposure is carried out using transmission lines only incident power is reported. Excellent techniques for electrical measurements of absorbed energy in a transmission line have been described [*Ho et al.*, 1973; *Christman et al.*, 1974]. However, these techniques have not been widely used presumably because of equipment expense and technical difficulty.

Calorimetric measurements of the energy absorbed by an exposed sample have been employed as a useful dosimetric method. Such measurements are based on the assumption that all absorbed energy is converted to heat within a short period of time instead of being stored or dissipated in some other form. A commonly used calorimetric method is the analysis of the rise of temperature of an exposed sample and its subsequent decrease following exposure. Earlier workers [*Jacobson and Süsskind*, 1958; *McRee*, 1974a,b; *Barthakur*, 1975] have used heating and cooling curves, but their techniques of analysis have not made the fullest use of the available data. In this paper we describe a simple analytical technique that fully utilizes all of the data from heating or cooling curves in order to estimate rate of energy absorption in a sample. The technique is applicable to all exposure conditions, and we have chosen as examples exposures in the far field and in a transmission line.

2. MATERIALS AND METHODS

Heating and cooling curves were recorded under two different methods of exposure. In the first method, far-field exposures were conducted at 2450 MHz CW in an anechoic room. The sample was placed in a Styrofoam environmental control chamber which regulated the temperature at 25 ± 0.5 °C. Details of this exposure system are provided in the literature [*Elder and Ali*, 1975; *Blackman et al.*, 1975]. The sample used was 40 ml of 0.15 M KCl solution contained in a plastic tissue culture flask (T-flask). A Yellow Springs Instruments type 520 solid-state thermistor probe was imbedded in the sample so that the tip of the probe was located approximately in the center of the flask. The T-flask was positioned so that its wide side faced the horn and the longer axis was parallel to the *E* field.

The presence of the thermistor did not materially affect the absorption of microwave energy by the sample since no temperature difference could be found between samples irradiated with and without the presence of the probe. Some pickup was noted at the measuring unit, but it was constant for a given experiment. It was attributed to signal pickup on the long length of unshielded thermistor lead in the anechoic chamber. This artifactual response was compensated for during analysis of the data.

The second exposure system involved a waveguide transmission-line system that was mounted in the sample compartment of a double-beam UV-visible spectrophotometer. The sample was irradiated by placing it inside a slotted waveguide applicator. This equipment has also been described in detail in the literature [*Allis et al.*, 1975; *Allis*, 1975]. Exposures were made with the equilibrium temperature of the sample controlled at 21 °C. In this case the sample consisted of 3 ml of 0.02 M KCl solution contained in a standard 1-cm^3 cross-section spectrophotometer cell. Temperature of the sample was measured by a microthermocouple installed through a frosted side of the spectrophotometer cell. No interference between the probe and the radiation was detected [*Allis et al.*, 1975].

In both experiments the output of the temperature sensor was amplified and recorded on a calibrated strip chart recorder. A continuous recording of a heating or cooling curve was obtained in less than an hour. The data were analyzed with a Hewlett Packard 98-30 programmable calculator equipped with a digitizer and

plotter. At least 70 to 80 points were obtained from each curve -- more than is normally feasible when performing hand calculations. With the program we were able to analyze as many as four competing exponential processes. The program is written in BASIC computer language and is available from the authors.

3. THEORY AND RESULTS

Heating and cooling curves can be analyzed to obtain calorimetric estimates of energy absorption by samples irradiated *in vitro* and, in some situations, *in vivo* [*Jacobson and Süsskind*, 1958; *Barthakur*, 1975; *McLees and Finch*, 1973]. Several methods of analysis have been suggested, including estimation of initial slopes [*Johnson*, 1975; *McRee*, 1974a] and use of cooling rates [*McRee*, 1974b]. These methods can be treated as solutions to a simple differential equation. This more general approach will cover situations where the system has attained the steady state as well as those situations where the system has not.

A system of mass m and heat capacity C that initially is at temperature T_0 is heated at a constant rate from time $t = 0$ to time $t = \tau$, when heating is terminated. The temperature at any time will reflect a balance between input and loss of thermal energy. If we assume that the input is constant until time $t = \tau$, and that for all t heat loss is proportional to the temperature difference between the system and its surroundings ($\Delta T = T - T_0$), we have for heating:

$$dT/dt = A - k\Delta T \qquad 0 \leqslant t \leqslant \tau \tag{1}$$

and for cooling

$$dT/dt = -k\Delta T \qquad t \geqslant \tau \tag{2}$$

where A is the rate of increase in temperature in the absence of losses and k is the proportionality constant for the rate of decrease of temperature (cooling rate constant). If the system is absorbing energy at a constant rate, W, then

$$W = mCA \tag{3}$$

which holds true for simple *in vitro* systems and for some *in vivo* systems.

Equations (1) and (2) may be directly integrated (using common tables of integrals) to yield

$$\Delta T = A/k\,(1 - e^{-kt}) \qquad 0 \leqslant t \leqslant \tau \tag{4}$$

$$\Delta T = \Delta T_\tau e^{-k(t-\tau)} \qquad t \geqslant \tau \tag{5}$$

where ΔT_τ is the temperature difference at the time when the heating is terminated. If the rate of temperature rise (without losses), A, can be evaluated, then the rate of energy absorption can be calculated from equation (3). In order to accomplish this without constraining the time, τ, when the heating is terminated, the cooling rate constant, k, must be first evaluated from the slope of a plot of equation (5), i.e., log ΔT versus time for $t > \tau$. The value of k so determined is then used with equation (4) and A is evaluated from the slope of a plot of ΔT versus e^{-kt}. This method has the disadvantage of requiring two plots to be made in order to obtain one value for the rate of energy absorption, W.

If the temperature of the system is allowed to reach a steady state, the rate of energy input equals the rate of loss and $\Delta T = \Delta T_{ss}$ = constant. Then from equation (4) we can find

$$\lim_{t \to \infty} \Delta T = \Delta T_{ss} = A/k \tag{6}$$

This relation can now be combined with equation (3) to obtain

$$W = mCk\Delta T_{ss} \tag{7}$$

Therefore, if the system reaches the steady state before the heat source is removed, the rate of energy absorption (or the mass-normalized specific absorption rate, SAR) can be determined solely from a plot of the cooling relation (equation (5)). A value for k will be obtained from the slope and the intercept will provide an additional estimate of ΔT_{ss}.

Alternatively, equation (6) can be used to eliminate A/k from equation (4) for the heating curve. After rearranging and taking logarithms we obtain

$$\log(\Delta T_{ss} - \Delta T) = -kt + \log \Delta T_{ss}, \qquad t \leqslant \tau. \tag{8}$$

Again, k may be obtained from the slope of a plot of $\log(\Delta T_{ss} - \Delta T)$ versus t, and the intercept will yield a value for ΔT_{ss}. The latter value must, of course, agree closely with that obtained from the raw data.

These relationships may now readily be used to develop the methods of analysis that have been suggested by other workers in this field. If equation (4) is differentiated with respect to time and evaluated at the limit $t = 0$, we have

$$d/dt\,(\Delta T) = Ae^{-kt}$$

$$\lim_{t \to 0} d/dt\,(\Delta T) = A \tag{9}$$

Therefore, if the data are sufficiently good to extract a usable value of the initial slope of the heating curve, W may be calculated [*Johnson*, 1975; *McRee*, 1974a].

The total energy absorbed, E, up to time τ can be expressed as $E = Wt$. Using this relation to substitute for t in equation (4), we obtain the equation

$$\Delta T = A/k\,(1 - e^{-E/W}) \tag{10}$$

This relation is essentially identical to the formulation of *Jacobson and Süsskind* [1958] and *McLees and Finch* [1973].

Representative heating and cooling curves obtained from the waveguide exposure system are illustrated in Figure 1. In each case the heating curves reach the steady state. Digitized data have been obtained from the continuous curves and treated according to the log form

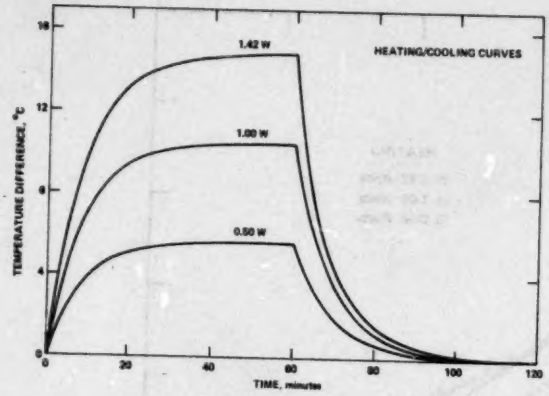

Fig. 1. Heating and cooling curves at three power levels taken from the sample exposed in the waveguide – spectrophotometer system. Curves were taken as continuous recordings.

of equation (5) for cooling and equation (8) for heating. Cooling data from these curves are illustrated in Figure 2 and heating data are illustrated in Figure 3.

The rate of energy absorption by a sample has been determined using heating and cooling curve data and equation (7). Such absorption measurements, obtained using the steady-state method, are illustrated in Figures 4 and 5 for two exposure systems. The SAR or rate of energy absorption in watts per kilogram (W/kg) has been plotted as a function of the relevant exposure parameter.

The data shown in Figure 4 were obtained from the waveguide exposure system. In this case the most convenient exposure parameter is generator output; the rate of energy absorption is presented as a function of this quantity. Both heating and cooling measurements are represented. The line is a least-squares fit to the data, and the intercept is less than one standard deviation from the origin. This curve provides calibration of the exposure system for a given set of experimental conditions. It only needs to be checked occasionally.

Figure 5 illustrates data from exposures in the anechoic chamber; the rate of energy absorption is plotted as a function of incident power density. Heating and cooling curves are represented at each power setting. The line is a least-squares fit to the data, which again extrapolates to within one standard deviation of the origin. This calibration plot can be used to estimate the rate of energy absorption for the sample at various incident power densities, provided the other exposure conditions remain unchanged.

The variability in the data of Figure 5 probably reflects to a large extent a) the difficulty in reproducibly repositioning the environmental control chamber within the anechoic room; and b) a variable field that is only con-

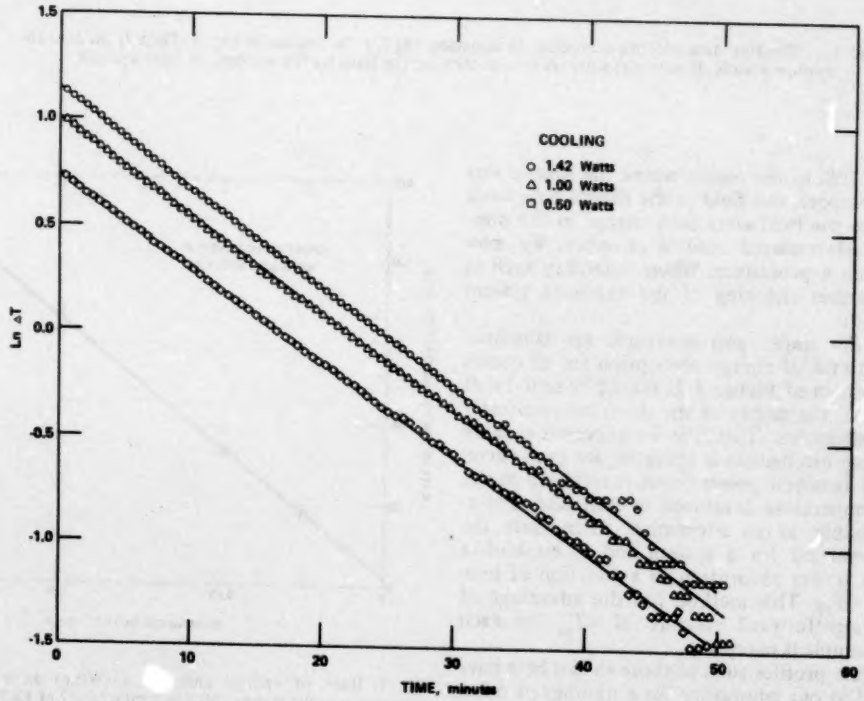

Fig. 2. Cooling data plotted according to equation (5) for the curves in Fig. 1. Data from zero to approximately 40 minutes were used to determine the lines by the method of least squares.

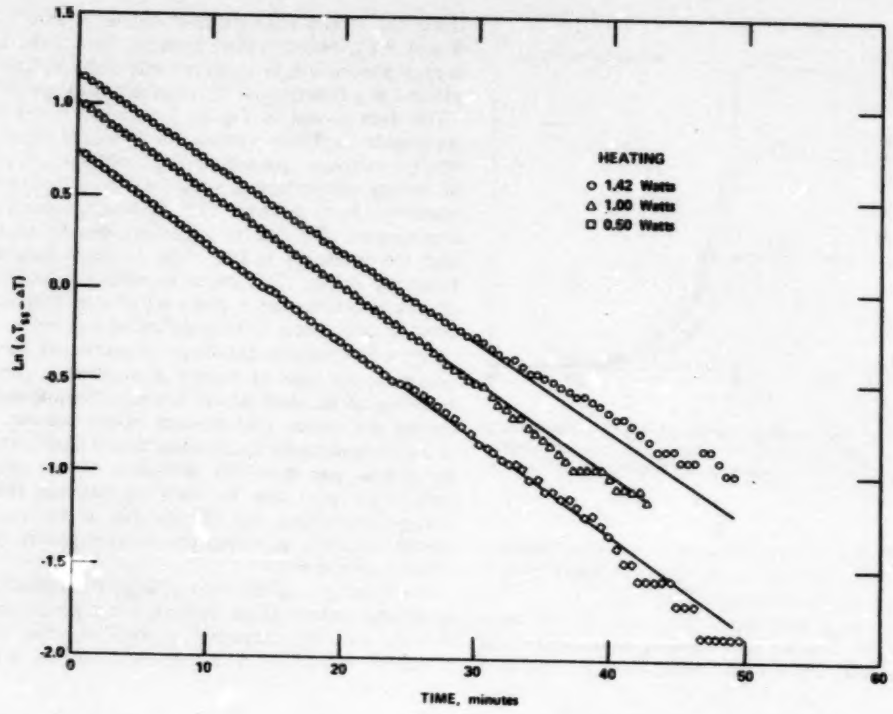

Fig. 3. Heating data plotted according to equation (8) for the curves in Fig. 1. Data from zero to approximately 35 minutes were used to determine the lines by the method of least squares.

stant within ± 10% in the region where the sample was located. In retrospect, our field probe should have been used to measure the field after each change in the position of the environmental control chamber. We now recommend such a procedure. When variability such as this occurs, further checking of the exposure system may be required.

In Table 1 the slopes and intercepts are tabulated along with the rate of energy absorption for all curves comprising the data of Figure 4. It should be noted that the values for k, the slopes of the lines, are essentially the same for all curves. This is to be expected because the same cooling mechanism is operating for each curve. The difference between power levels is reflected in the steady-state temperature developed in each case. Therefore, it is possible, as an alternative, to measure the cooling rate constant for a system and to establish a calibration for power absorption as a function of temperature rise, ΔT_{ss}. This method has the advantage of yielding a straightforward measure of ΔT_{ss} for each experimental sample if necessary.

Absorption-rate profiles such as those shown here have been measured in our laboratory for a number of other exposure geometries. These include the T-flask with smaller volumes of sample and several sizes of plastic petri dishes with different amounts of sample. In the

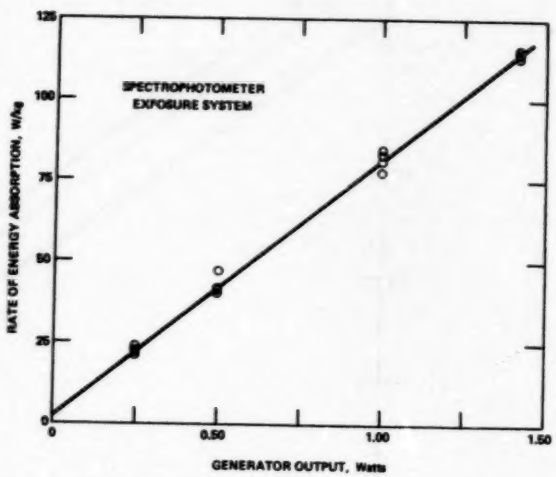

Fig. 4. Rate of energy absorption (W/kg) as a function of generator output power (W) for 3 ml of 0.02 M KCl exposed in a waveguide transmission line installed in the sample compartment of a double-beam spectrophotometer. Heating and cooling data are represented at each power density.

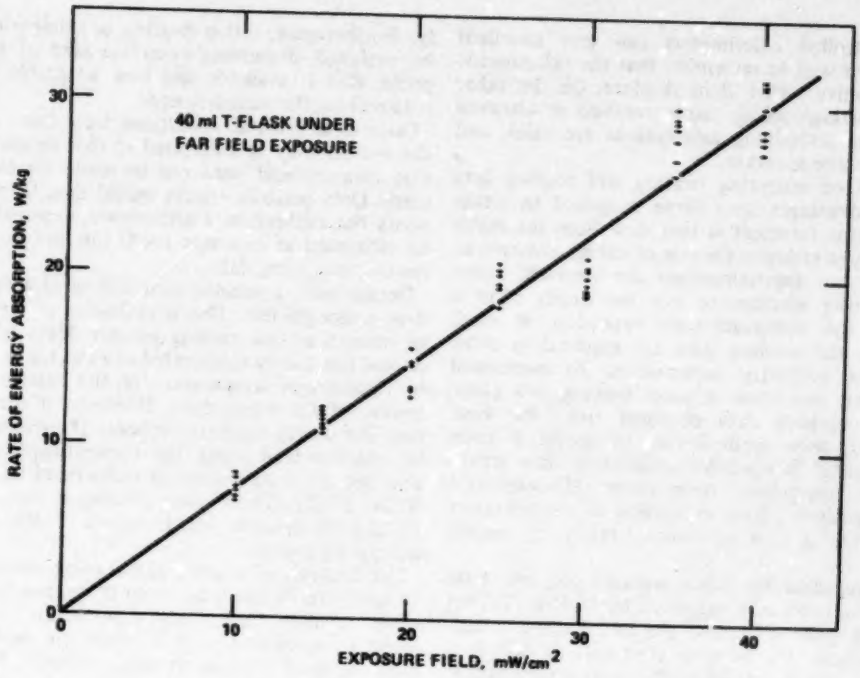

Fig. 5. Rate of energy absorption (W/kg) as a function of far-field power density (mW/cm^2) for a 40-ml T-flask filled with 0.15 M KCl. Heating and cooling data are represented at each power density.

latter case, it would found necessary to heat-seal the petri dishes in plastic wrap (Saran®) to limit evaporative cooling. These various measurements form the dosimetric basis for a number of experiments performed in the laboratory and described in the literature [*Allis*, 1975; *Allis et al.*, 1976; *Blackman et al.*, 1975, 1976; *Elder and Ali*, 1975; *Smialowicz*, 1976; *Ward et al.*, 1975].

TABLE 1. Cooling constants and steady-state temperature changes obtained from heating and cooling data.

Generator Output Watts	Heating/Cooling	-k, min^{-1}	ΔT_{ss}, °C	Absorption Rate W/kg
0.25	Heat	0.106	2.88	21.3
0.25	Cool	0.116	2.86	23.2
0.25	Heat	0.111	2.83	21.8
0.25	Cool	0.111	2.85	22.0
0.50	Heat	0.119	5.67	47.0
0.50	Cool	0.104	5.57	40.5
0.50	Heat	0.109	5.50	41.7
0.50	Cool	0.109	5.49	41.5
1.00	Heat	0.117	10.34	84.1
1.00	Cool	0.110	10.20	77.9
1.00	Heat	0.116	10.33	83.3
1.00	Cool	0.110	10.55	80.7
1.42	Heat	0.113	14.72	115.3
1.42	Cool	0.116	14.21	114.5
1.42	Heat	0.112	14.90	116.0
1.42	Cool	0.111	14.65	113.4

4. DISCUSSION

Analysis of heating and cooling curves provides a reliable and reproducible way of obtaining the rate of energy absorption of a sample exposed to microwave radiation, particularly samples exposed *in vitro*. The data obtained for the far-field exposure of filled T-flasks is in excellent agreement with that obtained by using the simple calorimetric method discussed in the accompanying paper [*Blackman and Black*, 1977]. These data should not, however, be taken as representative values for other exposure facilities. Each facility has its own characteristics and requires separate analysis.

In comparing this method of obtaining energy absorption to that utilizing calorimeters, it is important to remember that the heating/cooling curve method does not require calibration. All quantities in equation (7), for example, are directly obtainable from the experimental sample. In contrast, when using calorimeters the properties of the instrument must be calibrated with a known heat input. The calibration must be made for the simple equipment of *Blackman and Black* [1977] as well as the more sophisticated twin-well calorimeters described by *Phillips et al.*, [1975]. Furthermore, the sensitivity of the calorimeter depends upon a close match between the experimental sample and the calorimeter's wells. When these factors are

carefully controlled calorimeters can give excellent results, but it is well to remember that the calorimetric method is relative rather than absolute. On the other hand, the heating/cooling curve method is absolute as long as the underlying assumptions are valid, and is in principle more accurate.

Our method of analyzing heating and cooling data has several advantages over those proposed by other workers. Perhaps foremost is that data from the entire curve are used to estimate the rate of energy absorption, and therefore no approximations are involved. These points hold true whether or not the steady state is attained. If the nonsteady-state procedure is used, both heating and cooling data are required in order to obtain the necessary information. As mentioned previously, this procedure requires making two plots; the second requiring data obtained from the first. The nonsteady-state method can be useful if there are experimental limitations prohibiting the attainment of the steady-state temperature. However, the steady-state analysis allows evaluation of the necessary quantities from a plot of either heating or cooling data.

Methods suggested by other workers are less satisfactory. In the procedure suggested by *McRee* [1974a] for analyzing heating curves, the initial slope is estimated to obtain the heating constant, A, which is subsequently used to calculate the cooling constant, k. Evaluation of an initial slope is always somewhat subjective; furthermore, since A is obtained first, it can be used directly to calculate the rate of energy absorption. The analysis of cooling curves [*McRee*, 1974b] suggests evaluating k from two points on the curve. This places a very high reliance on a minimum amount of data when there are much more data available. The method discussed by *Johnson* [1975] – in which a temperature change is measured for a given period of time – depends upon the approximation that the early part of an exponential curve is linear. This approximation is valid if the steady-state ΔT that would ultimately develop is much larger than the measured ΔT. However, this condition may be difficult to meet, especially with low-powered generating equipment.

The slopes of the semilog plots are essentially constant for all power levels, as can be seen in Figures 2 and 3 and Table 1. A radical change in the slope (or k) can occur without a parallel change in the steady-state ΔT. However, this change indicates a change in the conditions of the experiment and such data should normally not be used. Such a variation in k becomes apparent readily when semilog plots are prepared. For example, one set of published heating-curve data [*McRee*, 1974b] shows such a variation when analyzed by our method, but apparently was not noticed by the author. For exposures at five power levels ranging from 20 mW/cm^2 to 200 mW/cm^2, cooling constants of -0.106, -0.095, -0.075, -0.102, and -0.105 were obtained using our analysis. The constant for the third exposure level is five standard deviations from the mean of the other four, and is of questionable utility.

Although our analysis was done using a computerized system, the method is simple enough to be done manually. Furthermore, either heating or cooling curves may be analyzed, depending upon the kind of temperature probe that is available and how adaptable the system is to making the measurement.

Once experimental conditions have been established, the system may be calibrated so that an energy-absorption measurement need not be made for every experiment. Only periodic checks would then be necessary to verify the calibration. Furthermore, doses of energy can be estimated at exposure levels too low to yield useful heating or cooling data.

Occasionally a semilog plot will yield a curved rather than a straight line. This is indicative of the occurrence of more than one cooling process. Most of the time a curved line can be interpreted as a change in the ambient or equilibrium temperature of the system during the course of the experiment. However, if there are truly two (or more) cooling mechanisms present, each may be characterized using the curve-stripping techniques that are commonly used in radiochemistry [*Wang and Willis*, 1965]. Evaporative cooling is one such source of heat dissipation which occurs if the samples are not tightly sealed.

The analysis of heating and cooling curves is difficult to apply to *in vivo* experiments because living animals often regulate their body temperature. Furthermore, there are problems with nonuniform heating in tissue even in dead or nonregulating animals. Placement of the probe in a hot or cool spot would yield a misleading measure of the rate of energy absorption. However, some workers have had success using the technique on *in vivo* systems. *Jacobson and Süsskind* [1958] used a heating-curve analysis to describe energy density in white mice exposed to 3-cm radiation. In a recent paper *Barthakur* [1975] has successfully obtained data for exposure of a leaf to nonionizing radiation. A recent review [*McLees and Finch*, 1973] discusses the potential for application of the method to comparative studies of animals.

As mentioned above, a limitation of the method for both *in vivo* and *in vitro* experiments is that unless there is uniform energy deposition within the sample, the value obtained will depend on where the probe is placed. In our far-field measurements, the probe was placed near the center of the sample and we obtained some average value for the dose. We attempted to check this value by thoroughly shaking the sample at the start of a cooling curve. Within the limits of this procedure, no difference was found between dose measurements on shaken and unshaken samples. For the exposures in the waveguide system, the temperature profile of the sample was determined [*Allis et al.*, 1975]. The measured dose was found to apply only to a limited portion of the sample. In any case, investigators must be aware that the measured dose applies only to the region near the probe and that further characterization may be required.

A potential disadvantage of the method is that increases in temperature of at least two or three degrees are needed in order to obtain reliable heating and cooling data. This requirement may be a problem if generating equipment is unable to produce such temperature

rises in the sample. One possible way to circumvent the problem is to enclose the sample in Styrofoam. The temperature rise will be substantially increased, and the associated cooling constant, k, will be reduced. The product of these values, and therefore the dose, will be the same as those without the Styrofoam, allowing for the small reflection and absorption of energy by this material. However, the addition of insulation may add another possible source of error since dose determination and exposure may not be done under identical conditions.

The presence of the probe in the sample did not appear to affect the measurement of dose for the two systems that are discussed in this paper. Both were checked by first irradiating the sample without the probe, then with the probe, and measuring a cooling curve for each case. For the far-field exposure of the T-flask, a simple calorimeter was used to get an independent estimation of absorbed energy [*Blackman and Black*, 1977]. Good agreement was found. We attribute these results to the small size and limited portion of thermocouple irradiated in the waveguide-exposure system; also, thermistors, as used for the far-field exposure, do not interact strongly with a microwave field [*McRee*, 1973]. It has also been recommended [*McRee*, 1973] that probes be placed perpendicular to the orientation of the E field in order to limit artifact. For exposure of the T-flasks in our study the probe was parallel to the E field, but a limited investigation with the probe perpendicular did not show any difference in dose. We would recommend, however, that the perpendicular arrangement be used whenever possible. Another approach would be to use one of the recently developed nonperturbing probes such as the liquid crystal type [*Cetas et al.*, 1976] or the high resistance type [*Bowman*, 1975].

In conclusion, we have described a method for obtaining a measure of energy absorption for samples irradiated in a microwave field. The method is particularly applicable to *in vitro* systems. It is our hope that all future biological experimentation in nonionizing radiation will involve measurements of energy absorption so that meaningful comparisons of results between laboratories can be made.

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